

# Prostate cancer risk by *BRCA2* genomic regions

Tommy Nyberg MSc, PhD candidate<sup>a,\*</sup>, Debra Frost ONC<sup>a</sup>, Daniel Barrowdale BSc<sup>a</sup>, D. Gareth Evans MD, PhD<sup>b</sup>, Elizabeth Bancroft RN<sup>c,d</sup>, Julian Adlard MD, PhD<sup>e</sup>, Munaza Ahmed MD, PhD<sup>f</sup>, Julian Barwell MBBS, PhD<sup>g</sup>, Angela F. Brady MD, PhD<sup>h</sup>, Carole Brewer MD, PhD<sup>i</sup>, Jackie Cook MD, PhD<sup>j</sup>, Rosemarie Davidson MD, PhD<sup>k</sup>, Alan Donaldson FRCP, BSc<sup>l</sup>, Jacqueline Eason MD, PhD<sup>m</sup>, Helen Gregory MD, PhD<sup>n</sup>, Alex Henderson MB, PhD<sup>o</sup>, Louise Izatt MA, MB, BChir, PhD<sup>p</sup>, M. John Kennedy MB, BCh<sup>q,r</sup>, Claire Miller MD, PhD<sup>s</sup>, Patrick J. Morrison MD, DSc<sup>t</sup>, Alex Murray MD, PhD<sup>u</sup>, Kai-Ren Ong MD, PhD<sup>v</sup>, Mary Porteous MD, PhD<sup>w</sup>, Caroline Pottinger MD, PhD<sup>x</sup>, Mark T. Rogers MD<sup>y</sup>, Lucy Side MD, PhD<sup>z</sup>, Katie Snape MD, PhD<sup>A</sup>, Vishakha Tripathi MB, BS, MSc<sup>p</sup>, Lisa Walker MD, PhD<sup>B</sup>, Marc Tischkowitz MD, PhD<sup>C</sup>, Rosalind Eeles MD, PhD<sup>c,d</sup>, Douglas F. Easton PhD<sup>a,†</sup>, Antonis C. Antoniou PhD<sup>a,†</sup>

<sup>a</sup> Centre for Cancer Genetic Epidemiology, Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom; <sup>b</sup> Manchester Regional Genetics Service, Central Manchester University Hospitals NHS Foundation Trust, Manchester, United Kingdom; <sup>c</sup> Oncogenetics Team, Division of Genetics and Epidemiology, The Institute of Cancer Research, London, United Kingdom; <sup>d</sup> Cancer Genetics Unit, Royal Marsden NHS Foundation Trust, London, United Kingdom; <sup>e</sup> Yorkshire Regional Genetics Service, Leeds Teaching Hospitals NHS Trust, Leeds, United Kingdom; <sup>f</sup> North East Thames Regional Genetics Service, Great Ormond Street Hospital for Children NHS Trust, London, United Kingdom; <sup>g</sup> Leicestershire Clinical Genetics Service, University Hospitals of Leicester NHS Trust, Leicester, United Kingdom; <sup>h</sup> North West Thames Regional Genetics Service, London North West University Healthcare NHS Trust, London, United Kingdom; <sup>i</sup> Peninsula Clinical Genetics Service, Royal Devon and Exeter NHS Foundation Trust, Exeter, United Kingdom; <sup>j</sup> North Trent Clinical Genetics Service, Sheffield Children's NHS Foundation Trust, Sheffield, United Kingdom; <sup>k</sup> West of Scotland Regional Genetics Service, NHS Greater Glasgow and Clyde, Glasgow, United Kingdom; <sup>l</sup> South Western Regional Genetics Service, University Hospitals Bristol NHS Foundation Trust, Bristol, United Kingdom; <sup>m</sup> Nottingham Centre for Medical Genetics, Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom; <sup>n</sup> North of Scotland Regional Genetics Service, NHS Grampian, Aberdeen, United Kingdom; <sup>o</sup> Northern Genetics Service, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle, United Kingdom; <sup>p</sup> South East Thames Regional Genetics Service, Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom; <sup>q</sup> St James's Hospital, Dublin, Republic of Ireland; <sup>r</sup> National Centre for Medical Genetics, Dublin, Republic of Ireland; <sup>s</sup> Merseyside and Cheshire Clinical Genetics Service, Liverpool Women's NHS Foundation Trust, Liverpool, United Kingdom; <sup>t</sup> Northern Ireland Regional Genetics Service, Belfast Health and Social Care Trust, Belfast, United Kingdom; <sup>u</sup> Medical Genetics Services for Wales, Abertawe Bro Morgannwg University Health Board, Swansea, United Kingdom; <sup>v</sup> West Midlands Regional Genetics Service, Birmingham Women's and Children's NHS Foundation Trust, Birmingham, United Kingdom; <sup>w</sup> South East of Scotland Regional Genetics Service, NHS Lothian, Edinburgh, United Kingdom; <sup>x</sup> Medical Genetics Services for Wales, Betsi Cadwaladr University Health Board, Bodelwyddan, United Kingdom; <sup>y</sup> All Wales Medical Genetics Service, NHS Wales, Cardiff, United Kingdom; <sup>z</sup> Wessex Clinical Genetics Service, University Hospital Southampton NHS Foundation Trust, Southampton, United Kingdom; <sup>A</sup> South West Thames Regional Genetics Service, St George's University Hospitals NHS Foundation Trust, London, United Kingdom; <sup>B</sup> Oxford Regional Genetics Service, Oxford University Hospitals NHS Foundation Trust, Oxford, United Kingdom; <sup>C</sup> Department of Medical Genetics, National Institute for Health Research Cambridge Biomedical Research Centre, University of Cambridge, Cambridge, United Kingdom

\* Corresponding author.

Address: Centre for Cancer Genetic Epidemiology, Department of Public Health and Primary Care, University of Cambridge, Strangeways Research Laboratory, Cambridge, CB1 8RN, United Kingdom.

Tel: +44 (0)1223 748646

Fax: +44 (0)1223 748628

Email address: [ten25@medschl.cam.ac.uk](mailto:ten25@medschl.cam.ac.uk)

† Contributed equally

**Keywords** *BRCA2*; genetic risk; genomic region; prospective cohort study; prostate cancer; prostate cancer cluster region

16<sup>th</sup> April 2020

Word count, Abstract: 150

Word count, body: 1227

## Abstract

A *BRCA2* prostate cancer cluster region (PCCR) was recently proposed (c.7914 to 3') wherein pathogenic variants (PVs) are associated with higher prostate cancer (PCa) risk than PVs elsewhere in the *BRCA2* gene. Using a prospective cohort study of 447 male *BRCA2* PV carriers recruited in the UK and Ireland from 1998 to 2016, we estimated standardised incidence ratios (SIRs) compared to population incidences and assessed variation in risk by PV location. Carriers of PVs in the PCCR had a PCa SIR of 8.33 (95% confidence interval [CI] 4.46-15.58) and were at higher risk of PCa than carriers of other *BRCA2* PVs (SIR=3.31, 95% CI 1.97-5.57; hazard ratio [HR]=2.34, 95% CI 1.09-5.03). PCCR PV carriers had an estimated cumulative PCa risk of 44% (95% CI 23%-72%) by age 75 and 78% (95% CI 54%-94%) by age 85. Our results corroborate the existence of a PCCR in *BRCA2* in a prospective cohort.

## Patient summary

In this report we investigated whether the risk of prostate cancer for men with a harmful mutation in the *BRCA2* gene differs based on where in the gene the mutation is located. We found that men with mutations in one region of *BRCA2* had a higher risk of prostate cancer than men with mutations elsewhere in the gene.

We recently reported prostate cancer (PCa) risk estimates for pathogenic variants (PVs) in *BRCA2*, based on a prospective cohort of male carriers [1]. Variability in cancer risks due to genotype-phenotype correlations may allow for more individualised counselling and screening. We noted that PVs within the so-called ovarian cancer cluster region (OCCR) in exon 11 of the gene [2–4] were associated with lower PCa risk than other *BRCA2* PVs [1,3,4]. PVs in the OCCR have been consistently shown to be associated with increased ovarian cancer risk but decreased breast cancer risk [2,3,5,6], although the precise boundaries of the OCCR [3,5] and the mechanisms behind this risk variation remain uncertain. It has been proposed that the likelihood that a PV triggers nonsense-mediated mRNA decay varies by genomic region [7,8], so that OCCR PVs might produce a truncated or alternatively spliced protein whose capability to suppress tumours varies by cancer type [2,3,5,7,8], but there is currently no experimental support for this hypothesis [7]. Shortly after the publication of our manuscript, Patel and co-workers proposed the existence of a prostate cancer cluster region (PCCR) at the 3' end of *BRCA2*, based on retrospective cohort data [8]. This retrospective study reported that men with *BRCA2* PVs in the proposed PCCR have a higher risk of PCa (hazard ratio [HR]=1.78, 95% confidence interval [CI] 1.25-2.52), particularly Gleason score $\geq$ 8 PCa (HR=3.11, 95% CI 1.63-5.95), compared to men with PVs in the reference region c.1001 to c.7913, but did not present estimates of the absolute PCa risk for PCCR PV carriers [8]. In order to substantiate or refute this association, and to provide direct estimates of the absolute risk of PCa for carriers of *BRCA2* PCCR PVs, we have reanalysed our prospective data.

The prospective cohort comprised 447 male *BRCA2* PV carriers who were recruited to the EMBRACE study (<http://ccge.medschl.cam.ac.uk/embrace/>) through clinical genetics centres in the UK and Ireland between 1998 and 2016 at median age of 51.4 yr (inter-quartile range 41.5-63.6 yr). The participants were counselled with regard to their PV. Detailed information on the cohort and on inclusion criteria, data collection, follow-up, and statistical analysis approach, is available in our recent publication [1]. The participants' PVs (listed in Supplementary table 1) were grouped on the basis of position within the *BRCA2* gene, based on the proposed PCCR (c.7914 to 3' [8]; HGVS nomenclature [<http://varnomen.hgvs.org/>]; using cDNA reference sequence NM\_000059.3 and reference genome hg18) and the wide definition of the OCCR (c.2831 to c.6401) [1–4]. We additionally considered the region bounded by c.756 and c.1000 in which Patel and co-workers found evidence of increased PCa risk [8], but due to a small sample size (n=1) we could not estimate the PCa risk associated with this region. Here, we also present floating absolute risks (FARs) [9] to enable risk comparisons between any of the considered genomic regions.

The Anglia and Oxford Medical Research and Ethics Committee approved the study. All participants provided written informed consent.

Twenty-six participants developed PCa during median follow-up of 5.3 yr (inter-quartile range 2.6-8.9 yr) [1]. Carriers of PVs in the PCCR (n=93) had a PCa standardised incidence ratio (SIR) of 8.33 (95% CI 4.46-15.6), whereas carriers of PVs elsewhere in *BRCA2* (n=354) had an SIR of 3.31 (95% CI 1.97-5.57) compared to population incidences. This corresponds to a significantly higher PCa risk associated with PVs in the PCCR compared to non-PCCR PVs (HR=2.34, 95% CI 1.09-5.03; Table 1). Compared to PVs in the region c.1001 to c.7913 [8], PCCR PVs were associated with a HR of 2.09 (95% CI 0.98-4.45). As previously reported, the SIR for carriers of PVs in the wide definition OCCR

(n=178) was 2.46 (95% 1.07-5.64) [1], and the risk for carriers of PCCR PVs was also significantly higher than that for OCCR PV carriers (HR=3.41, 95% CI 1.27-9.16). The SIR for PVs located in the region bounded by the OCCR and the PCCR (c.6402 to c.7913; n=66) was estimated to be 6.14 (95% CI 2.18-17.3), and the SIR for *BRCA2* PVs upstream of the OCCR (5' to c.2830; n=108) was 3.50 (95% CI 1.48-8.26). The FARs for the comparison of risks across the four regions suggested that the observed increased risk associated with PVs in the PCCR may be partly driven by the lower risk associated with PVs in the OCCR (Table 1). The proportional hazards assumption was violated for the model with all genomic regions fitted (Schoenfeld residuals test, p=0.003); in line with this the corresponding Kaplan—Meier plot indicated that the risks might be similar between the OCCR and PCCR PV carriers at younger ages but deviate at older ages. PCCR PV carriers had an estimated cumulative PCa risk of 44% (95% CI 23%-72%) by age 75 and 78% (95% CI 54%-94%) by age 85. After omitting the first six mo of follow-up to assess the possible effect of screening-associated diagnoses of indolent PCas, the corresponding estimates were 41% (95% CI 20%-73%) and 69% (95% CI 42%-91%), respectively (Figure 1).

The difference in PCa risk for PVs in PCCR vs OCCR remained statistically significant after adjusting for family history of PCa (number of first- and second-degree relatives diagnosed with PCa; adjusted HR=3.00, 95% CI 1.06-8.54) or geographical location (adjusted HR=3.79, 95% CI 1.41-10.2). This difference remained similar after omitting related individuals (HR=4.29, 95% CI 1.30-14.2), after omitting the first six mo of follow-up (HR=3.96, 95% CI 1.18-13.3), and after omitting carriers of PVs in the region c.756 to c.1000 (HR=3.42, 95% CI 1.27-9.18) or missense variants (HR=3.76, 95% CI 1.36-10.4). When carriers of the Ashkenazi founder PV c.5946delT (n=42) which is located in the OCCR was omitted, the difference in PCa risk between PCCR and OCCR PV carriers was not statistically significant but the HR estimate was of similar magnitude (HR=2.89, 95% CI 0.98-8.53; Supplementary table 2).

We did not observe a higher risk of Gleason score $\geq$ 8 PCa for PVs in the PCCR compared to non-PCCR PVs (HR=0.87, 95% CI 0.12-6.34), or compared to PVs in the region c.1001 to c.7913 (HR=0.79, 95% CI 0.11-5.69). However, the HRs did not differ significantly from those for Gleason score $\leq$ 7 PCa (PCCR vs non-PCCR: HR=3.32, 95% CI 1.25-8.84; test for heterogeneity, p=0.052; PCCR vs c.1001 to c.7913: HR=2.94, 95% CI 1.11-7.80; test for heterogeneity, p=0.088).

Our results corroborate the observation that carriers of PVs in the PCCR of the *BRCA2* gene [8] are at a higher risk of PCa than other *BRCA2* PV carriers. Patel and co-workers reported a HR of 1.78 (95% CI 1.25-2.52) compared to PVs in the region c.1001 to c.7913 [8], consistent with our HR estimate of 2.09 (95% CI 0.98-4.45). Our findings do not support a stronger association with a more aggressive phenotype, but these estimates were based on a small number of cases and have wide CIs. PV carriers may receive enhanced screening which may lead to biases in comparisons against the population incidence [1]. However, current screening practices do not differ by *BRCA2* PV location and so this is unlikely to have confounded the comparisons between the *BRCA2* genomic regions. A much larger cohort of unaffected carriers with longer follow-up is required to provide more precise PV-specific risk estimates, and to further clarify whether the observed variation in risk reflects lower risks associated with PVs outside the OCCR and PCCR than the risk associated with PCCR PVs, or solely a lower risk associated with PVs in the OCCR.

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## Figure and Table legends

### Figure 1

Absolute prostate cancer risk, (A) by location of *BRCA2* pathogenic variant, (B) by location of *BRCA2* pathogenic variant and with follow-up initiated six mo after study entry.

The number at risk at each age is shown above the x-axis. The curves are truncated at ages when fewer than five participants are at risk.

**Abbreviations** OCCR: ovarian cancer cluster region; PCCR: prostate cancer cluster region.

### Table 1

Prostate cancer risk by location of *BRCA2* pathogenic variant.

**Abbreviations** SIR: standardised incidence ratio; CI: confidence interval; HR: hazard ratio; FAR: floating absolute risk; PV: pathogenic variant; OCCR: ovarian cancer cluster region; PCCR: prostate cancer cluster region.

## 207 Acknowledgements

208

209 We thank all the participants in the EMBRACE study. This work was supported by Cancer Research  
210 UK grants C12292/A20861 and C12292/A22820. EMBRACE was supported by Cancer Research UK  
211 grants C1287/A23382 and C1287/A26886. D. Gareth Evans is supported by a National Institute for  
212 Health Research grant to the Biomedical Research Centre, Manchester (IS-BRC-1215-20007).  
213 Rosalind Eeles is supported by Cancer Research UK grant C5047/A8385, and by National Institute for  
214 Health Research support to the Biomedical Research Centre at The Institute of Cancer Research and  
215 The Royal Marsden NHS Foundation Trust.

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## 217 Conflict of interest statement

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219 All authors declare that they have no conflict of interest.

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## 221 Data sharing statement

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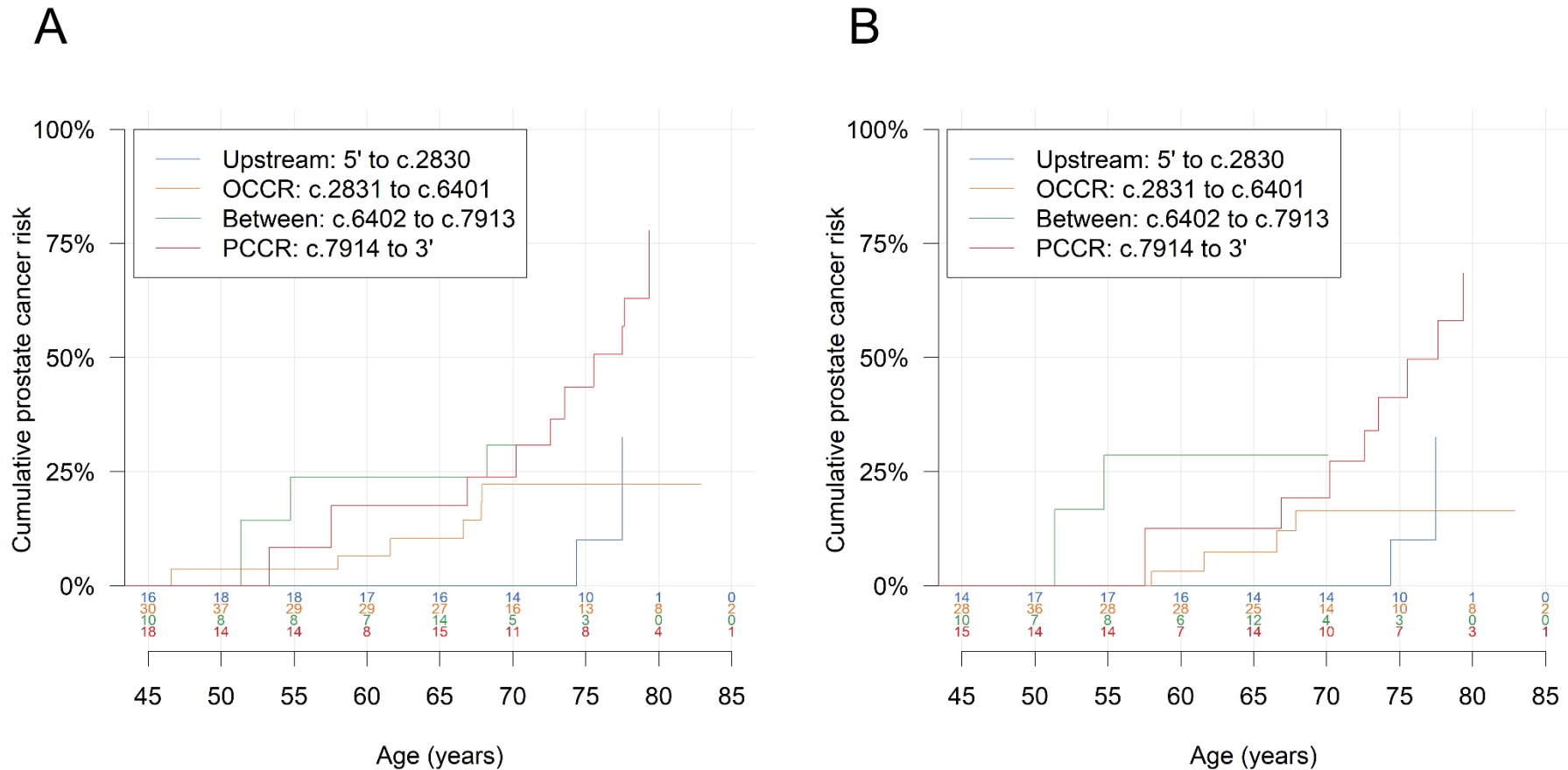
223 The data used in the analysis are available to other researchers upon request to the EMBRACE study  
224 coordinators (<https://ccge.medschl.cam.ac.uk/embrace/>).

225

**Figure 1** Absolute prostate cancer risk, (A) by location of *BRCA2* pathogenic variant, (B) by location of *BRCA2* pathogenic variant and with follow-up initiated six mo after study entry.

The number at risk at each age is shown above the x-axis. The curves are truncated at ages when fewer than five participants are at risk.

**Abbreviations** OCCR: ovarian cancer cluster region; PCCR: prostate cancer cluster region.





**Table 1** Prostate cancer risk by location of *BRCA2* pathogenic variant.

**Abbreviations** SIR: standardised incidence ratio; CI: confidence interval; HR: hazard ratio; FAR: floating absolute risk; PV: pathogenic variant; PCCR: prostate cancer cluster region; OCCR: ovarian cancer cluster region.

PV location	N	Person-years	Observed events	Incidence rate per 1000 person-years (95% CI)	Expected events	SIR (95% CI)	HR (95% CI)	FAR (95% CI)
<b>Compared to non-PCCR PVs</b>								
Non-PCCR (5' to c.7913)	354	2029.8	15	7.39 (4.45-12.3)	4.53	3.31 (1.97-5.57)	Reference	
PCCR (c.7914 to 3')	93	524.6	11	21.0 (11.4-38.7)	1.32	8.33 (4.46-15.6)	2.34 (1.09-5.03)	
<b>Compared to OCCR PVs</b>								
5' to c.2830	108	625.8	5	7.99 (3.37-19.0)	1.43	3.50 (1.48-8.26)	1.72 (0.50-5.94)	1.72 (0.70-4.24)
OCCR (c.2831 to c.6401) <sup>i</sup>	178	1054.4	6	5.69 (2.54-12.8)	2.44	2.46 (1.07-5.64)	Reference	1.00 (0.43-2.33)
c.6402 to c.7913	66	338.8	4	11.8 (4.29-32.5)	0.65	6.14 (2.18-17.3)	3.23 (0.79-13.2)	3.23 (1.15-9.11)
PCCR (c.7914 to 3')	93	524.6	11	21.0 (11.4-38.7)	1.32	8.33 (4.46-15.6)	3.41 (1.27-9.16)	3.41 (1.96-5.95)
Indeterminable	2							

<sup>i</sup> Detailed results for carriers of PVs in the OCCR are available in a previous publication [1].

**Supplementary table 1** List of *BRCA2* pathogenic variants carried by the 447 study participants.

The pathogenic variants are specified using HGVS nomenclature (<http://varnomen.hgvs.org/>), using cDNA reference sequence NM\_000059.3 and reference genome hg18.

**Abbreviations** DL: large deletion; S: splice site.

Genomic level	Protein level	n
c.-227-?_67+?del	p.0	6
c.-227-?_6841+?del	p.0	1
c.17_18del	p.Lys6fs	1
c.22_23del	p.Arg8fs	2
c.26del	p.Pro9fs	5
c.36dup	p.Glu13*	1
c.104_110del	p.Leu35fs	2
c.314T>G	p.Leu105*	1
c.396T>A	p.Cys132*	1
c.407del	p.Asn136fs	2
c.470_474del	p.Lys157fs	1
c.516+1G>T	S	1
c.517-2A>G	S	2
c.538_539dup	p.Ser181fs	2
c.574_575del	p.Met192fs	3
c.631+1G>A	S	1
c.631+2T>G	p.Gly173fs	5
c.658_659del	p.Val220fs	4 <sup>i</sup>
c.755_758del	p.Asp252fs	19
c.765_770delinsAAACAAT	p.Asn255fs	1
c.1097T>G	p.Leu366*	1
c.1189_1190insTTAG	p.Gln397fs	7
c.1231del	p.Ile411fs	1
c.1310_1313del	p.Lys437fs	3
c.1654del	p.Ser552fs	1
c.1689G>A	p.Trp563*	3
c.1787_1799del	p.Asp596fs	2
c.1813del	p.Ile605fs	3
c.1813dup	p.Ile605fs	4
c.1889del	p.Thr630fs	1
c.1929del	p.Arg645fs	7
c.2409T>G	p.Tyr803*	3
c.2606C>G	p.Ser869*	1
c.2701del	p.Ala902fs	1
c.2760del	p.Ile921fs	1
c.2808_2811del	p.Ala938fs	10
c.2870del	p.Asn957fs	1
c.3009_3010del	p.His1003fs	1
c.3158T>G	p.Leu1053*	2
c.3195_3198del	p.Asn1066fs	2

Genomic level	Protein level	n
c.3405C>A	p.Tyr1135*	1
c.3530_3533del	p.Asp1177fs	1
c.3545_3546del	p.Phe1182*	2
c.3599_3600del	p.Cys1200*	1
c.3680_3681del	p.Leu1227fs	1
c.3785C>G	p.Ser1262*	4
c.3847_3848del	p.Val1283fs	4
c.3860del	p.Asn1287fs	1
c.4037_4038del	p.Thr1346fs	1
c.4101del	p.Lys1367fs	1
c.4137_4141del	p.Ile1380fs	1
c.4163_4164delinsA	p.Thr1388fs	5
c.4169del	p.Leu1390fs	1
c.4223del	p.Gln1408fs	1
c.4405_4409del	p.Asp1469fs	1
c.4415_4418del	p.Lys1472fs	1
c.4478_4481del	p.Glu1493fs	10
c.4525C>T	p.Gln1509*	1
c.4631dup	p.Asn1544fs	1
c.4638del	p.Phe1546fs	1
c.4648G>T	p.Glu1550*	1
c.4712_4713del	p.Glu1571fs	2
c.4828dup	p.Val1610fs	1
c.4876_4877del	p.Asn1626fs	4
c.4889C>G	p.Ser1630*	2
c.4914dup	p.Val1639fs	1
c.4936_4939del	p.Glu1646fs	1
c.4981del	p.Tyr1661fs	1
c.5073dup	p.Trp1692fs	4
c.5116_5119del	p.Asn1706fs	3
c.5141_5144del	p.Tyr1714fs	1
c.5217_5223del	p.Tyr1739*	1
c.5217T>A	p.Tyr1739*	1
c.5279C>G	p.Ser1760*	2
c.5298del	p.Asn1766fs	1
c.5303_5304del	p.Leu1768fs	2
c.5329_5334delinsG	p.Lys1777fs	1
c.5350_5351del	p.Asn1784fs	2
c.5410_5411del	p.Val1804fs	1
c.5576_5579del	p.Ile1859fs	4
c.5641_5644del	p.Lys1881fs	1
c.5655C>A	p.Cys1885*	1
c.5682C>G	p.Tyr1894*	10
c.5722_5723del	p.Leu1908fs	2
c.5835dup	p.Ser1946fs	1

Genomic level	Protein level	n
c.5857G>T	p.Glu1953*	1
c.5909C>A	p.Ser1970*	6
c.5946del	p.Ser1982fs	42
c.6049A>T	p.Lys2017*	1
c.6052_6053del	p.Ser2018*	1
c.6065C>G	p.Ser2022*	1
c.6079dup	p.Arg2027fs	1
c.6081dup	p.Glu2028fs	1
c.6275_6276del	p.Leu2092fs	27 <sup>i</sup>
c.6385G>T	p.Glu2129*	1
c.6405_6409del	p.Asn2135fs	1
c.6486_6489del	p.Lys2162fs	1
c.6588_6589del	p.Lys2196fs	1
c.6591_6592del	p.Glu2198fs	5
c.6602del	p.Ser2201fs	1
c.6658_6662del	p.Glu2220fs	1
c.6757_6758del	p.Leu2253fs	2
c.6829_6833del	p.Ile2278fs	1
c.6944_6947del	p.Ile2315fs	4
c.6980del	p.Leu2327*	3
c.6996_7004delins(20)	p.Cys2332fs	3
c.7008-?_7805+?del	DL	13
c.7008-?_8331+?del	DL	1
c.7069_7070del	p.Leu2357fs	5
c.7342_7343del	p.Lys2448fs	1
c.7480C>T	p.Arg2494*	4
c.7495C>T	p.Gln2499*	1
c.7543dup	p.Thr2515fs	1
c.7558C>T	p.Arg2520*	1
c.7757G>A	p.Trp2586*	6
c.7758G>A	p.Trp2586*	2
c.7762_7764delinsTT	p.Ile2588fs	4
c.7795G>T	p.Glu2599*	1
c.7884dup	p.Trp2629fs	3
c.7934del	p.Arg2645fs	1
c.7958T>C	p.Leu2653Pro	1
c.7977-1G>C	S	4
c.7977-2_-3del	S	1
c.7988A>T	p.Glu2663Val	2
c.8113dup	p.Ser2705fs	1
c.8167G>C	p.Asp2723His	6
c.8247_8248del	p.Lys2750fs	2
c.8297del	p.Thr2766fs	9
c.8395del	p.Arg2799fs	1
c.8575del	p.Gln2859fs	9

Genomic level	Protein level	n
c.8633-?_8754+?del	DL	1
c.8633-?_9256+?del	DL	2
c.8756del	p.Gly2919fs	3
c.8878C>T	p.Gln2960*	1
c.8904del	p.Val2969fs	9
c.8945_8946del	p.Lys2982fs	1
c.8951C>G	p.Ser2984*	3
c.8956dup	p.Ile2986fs	1
c.9054_9055del	p.Ser3018fs	4
c.9069_9076del	p.Asn3024fs	2
c.9097dup	p.Thr3033fs	2
c.9117+1G>A	S	1
c.9117G>A	p.Val2985fs	2
c.9157del	p.Glu3053fs	2
c.9253dup	p.Thr3085fs	2
c.9257-2A>G	S	1
c.9294C>G	p.Tyr3098*	6
c.9357_9360del	p.Ile3120fs	2
c.9380G>A	p.Trp3127*	1
c.9382C>T	p.Arg3128*	7
c.9481A>T	p.Lys3161*	1
c.9490_9491del	p.Asn3164fs	1
c.9502-2A>C	S	1

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<sup>i</sup> One participant carried both c.658\_659del and c.6275\_6276del.

**Supplementary table 2** Prostate cancer risk by location of *BRCA2* pathogenic variant: adjustments and sensitivity analyses.

**Abbreviations** HR: hazard ratio; CI: confidence interval; PV: pathogenic variant; PCCR: prostate cancer cluster region; OCCR: ovarian cancer cluster region.

PV location	HR (95% CI)	HR adjusted for family history <sup>i</sup> (95% CI)	HR adjusted for geographical location <sup>ii</sup> (95% CI)	HR omitting related participants <sup>iii</sup> (95% CI)	HR omitting the first 6 mo of follow-up (95% CI)	HR omitting carriers of PVs in c.756 to c.1000 <sup>iv</sup> (95% CI)	HR omitting missense variant carriers <sup>v</sup> (95% CI)	HR omitting Ashkenazi founder PV carriers <sup>vi</sup> (95% CI)
<b>Compared to non-PCCR PVs</b>								
Non-PCCR (5' to c.7913)	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
PCCR (c.7914 to 3')	2.34 (1.09-5.03)	2.03 (0.89-4.61)	2.50 (1.15-5.46)	2.93 (1.23-6.97)	2.23 (0.93-5.37)	2.33 (1.09-5.01)	2.56 (1.17-5.59)	2.05 (0.94-4.48)
<b>Compared to OCCR PVs</b>								
5' to c.2830	1.72 (0.50-5.94)	1.77 (0.53-5.98)	1.86 (0.47-7.36)	1.60 (0.34-7.49)	2.55 (0.63-10.3)	1.75 (0.51-6.03)	1.77 (0.51-6.17)	1.49 (0.40-5.62)
OCCR (c.2831 to c.6401)	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
c.6402 to c.7913	3.23 (0.79-13.2)	2.86 (0.65-12.7)	4.18 (0.96-18.2)	3.65 (0.67-19.7)	4.03 (0.75-21.7)	3.24 (0.80-13.2)	3.21 (0.79-13.0)	2.77 (0.61-12.5)
PCCR (c.7914 to 3')	3.41 (1.27-9.16)	3.00 (1.06-8.54)	3.79 (1.41-10.2)	4.29 (1.30-14.2)	3.96 (1.18-13.3)	3.42 (1.27-9.18)	3.76 (1.36-10.4)	2.89 (0.98-8.53)
Indeterminable								

<sup>i</sup> Number of first- and second-degree relatives diagnosed with prostate cancer.

<sup>ii</sup> Location of recruiting clinic: London; South or East England; Wales, English Midlands or North England; Scotland or Ireland.

<sup>iii</sup> Carriers for which at least one male relative was included in the study (n=94 omitted).

<sup>iv</sup> Carriers of PVs in the *BRCA2* region c.756 to c.1000 suggested to be associated with increased PCa risks by Patel et al (2020) (n=1 omitted).

<sup>v</sup> Carriers of pathogenic missense variants (n=9 omitted).

<sup>vi</sup> Carriers of c.5946delT (n=42 omitted).